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**Misdiagnosis and uncritical use of plant mycorrhizal data are not the only elephants in the room: A response to Brundrett & Tedersoo (2018) 'Misdiagnosis of mycorrhizas and inappropriate recycling of data can lead to false conclusions'.**

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**Misdiagnosis and uncritical use of plant mycorrhizal data are not the only elephants in the room: A response to Brundrett & Tedersoo (2018) ‘Misdiagnosis of mycorrhizas and inappropriate recycling of data can lead to false conclusions’.**

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There is increasing interest in using plant mycorrhizal traits – characteristics related to a plant's ability to form mycorrhizal symbiosis – to understand the role of mycorrhizas within and across communities, ecosystems and biogeographical regions (Moora, 2014; Tedersoo, 2017). Recent studies incorporating plant mycorrhizal traits, mainly mycorrhizal type (e.g. ecto-, ericoid-, arbuscular-, and non-mycorrhizal; ECM, ERM, AM, NM) and mycorrhizal status (obligately and facultatively mycorrhizal; OM, FM; e.g. Correia et al. 2018; Gerz et al. 2018), have assigned trait values from published empirical data for plant species of interest. Dedicated efforts to build and improve databases of empirical plant mycorrhizal traits (e.g. Chaudhary et al. 2016; Bueno et al. 2017) are making these resources increasingly comprehensive, transparent and accessible.

A recent viewpoint paper by Brundrett and Tedersoo (2019) highlights several challenges connected with assigning mycorrhizal traits to plant species and criticizes the use of mycorrhizal trait databases. The authors argue that the use of such trait databases (which they refer to as “recycled data”) is ‘inappropriate’ due to two underappreciated problems: the databases (i) may include ‘mycorrhizal trait allocation errors’ due to misidentification of root mycorrhizal structures; or (ii) may contain data derived using diagnostic criteria that are flawed. They propose instead (1) diagnostic criteria for defining mycorrhizas that make specific assumptions about the biology of the interaction, and (2) checking databases against their curated list, derived from prior experience of assigning mycorrhizal traits to plant species (hereafter referred to as a 'standard reference', Brundrett, 2009; Tedersoo, 2017). Although we fully agree that standard protocols are required, there are several questions raised by the Brundrett and Tedersoo (2019) viewpoint that deserve critical consideration if consensus is to be reached among researchers in the field: 1) should the presence of minimum number of arbuscules define the AM symbiosis; 2) does plant taxonomy accurately predict plant mycorrhizal traits (i.e. high phylogenetic trait conservatism); 3) should the results of published studies be considered ‘incorrect’ when they do not match this, or any proposed, ‘standard reference’?

Below, we discuss these three questions and address an additional topic that arises from consideration of Brundrett and Tedersoo's criticisms of prior research: 4) the level of

transparency involved in building plant mycorrhizal trait databases and directions for future research.

#### **Point 1. Should the presence of arbuscules define AM plants?**

Symbiosis describes any intimate association of two organisms, while mutualism is an association that provides benefits for both. Mycorrhizal symbiosis is often interpreted as a mutualism, but depending on the environmental conditions in nature, the association varies along a continuum from mutualism to parasitism (Johnson *et al.*, 1997; Johnson & Graham, 2013). The nutritional benefit of mycorrhizal interactions to plants has received most attention, but non-nutritional benefits, such as improved biotic and abiotic stress tolerance for host plants (Delavaux *et al.*, 2017) and habitat for the fungal partner (Brundrett, 2002), can occur and are also potential drivers of the symbiosis. Thus, in our opinion, the criterion proposed by Brundrett and Tedersoo, which focuses solely on the nutritional exchange, is too limited. Furthermore, Brundrett and Tedersoo argue that AM plant species should be defined by mycorrhizal structures devoted to nutrient exchange: the presence of arbuscules. While the presence of arbuscules could indicate P-transfer (if arbuscules are functional), the absence of arbuscules does not necessarily mean that nutrients are not transferred. For example, nutrient exchange in AM also occurs through hyphal coils (Paris colonization type) with no arbuscules involved (Dickson, 2004), or even in the absence of coils and arbuscules (Manjarrez *et al.*, 2010). Besides, the lifetime of arbuscules is short, and their presence depends on plant developmental stage (Montero & Paszkowski, 2018), meaning that detection of arbuscules is context dependent and practically challenging (Vierheilig *et al.*, 2005). Therefore, the distinction between AM plants (with arbuscules) and non-mycorrhizal (NM) plants with 'Glomeromycotan Fungal Colonization' (with no arbuscules or non-functional ones) based on potential nutritional function or lack thereof (Brundrett and Tedersoo 2019), is inconsistent with published observations. Also, any criterion that is based on terms such as "few", "many" and "low" (Table 1, Brundrett and Tedersoo 2019) is subjective. Based on this, we think it is premature to use the definition of AM proposed by Brundrett and Tedersoo 2019 and advocate for the more inclusive morphological criterion previously proposed by Smith & Read (2008), which is based only on root intracellular colonization by glomeromycotinan fungi. This definition is widely applicable to the different

contexts that occur in nature (e.g., during development of mycorrhizal fungal colonization, plant-specific developmental stages, seasonal and soil fertility variation) and avoids any criteria linked to a function (i.e., nutritional, non-nutritional) of the symbiosis, where the degree of mutualism may fluctuate independently from morphological structures. Moreover, in ECM plants, N-transfer does not necessarily require a Hartig net (Sa *et al.*, 2019). This demonstrates that for ECM symbiosis there are potential new discoveries that may change our understanding about the functioning of mycorrhizal symbiosis. It is our hope, however, that a definition encompassing all the complexities of the mycorrhizal symbiosis will evolve as our knowledge advances.

## **Point 2. Can plant taxonomy be a reliable predictor of plant mycorrhizal traits?**

Brundrett and Tedersoo proposed a ‘standard reference’ for checking whether any new list of plant mycorrhizal traits contains potential errors. This ‘standard reference’ approach is based, in part, on the assumption that mycorrhizal traits can be assigned to species by extrapolating from higher taxonomic units, such as families or genera. The approach relies on the expectation that plant mycorrhizal traits are phylogenetically conserved within plant families or genera to a degree that allows predictions to be made. However, this assumption is not always justified. Taxonomy based extrapolations at lower taxonomic levels (e.g. within families and genera) may be more suitable for some mycorrhizal types (ECM, ERM, ORM), where well-studied plant groups are expected to exhibit highly conserved mycorrhizal traits. However, the approach could even yield errors in these groups because assumptions about the uniformity of mycorrhizal traits within well-studied plant groups are regularly disproved. For example, the ECM association was unexpectedly documented (based on morphology) in *Pulsatilla patens* (Hoeksema *et al.*, 2018), a species belonging to a family (Ranunculaceae) that contains a number of species, including *P. patens*, that have been shown experimentally to be highly dependent on AM symbiosis (Moora *et al.*, 2004).

A recent comparison of the Brundrett and Tedersoo’s ‘standard reference’ and literature-derived databases of European plant species indicated frequently diverging mycorrhizal trait assignment. Based on the literature databases, only 19% and 6% of plant families (out of 75

European plant families with more than five studied species) comprised a single plant mycorrhizal type or status, respectively (Bueno *et al.*, 2019). One cause for low phylogenetic conservatism in mycorrhizal traits is that some plant species have adapted to different environmental conditions in a way that elicits changes in mycorrhizal traits (Gerz *et al.*, 2018). For instance, Osborne *et al.* (2018) recently described how adaptation to different soil conditions was accompanied by a divergence of mycorrhizal traits in sister palm species. This type of adaptation has not been systematically explored, but it may explain why phylogeny is not a consistently reliable tool for determining plant mycorrhizal traits within plant families or genera.

Given the discrepancies noted by Bueno *et al.* (2019) and the limited volume of existing plant mycorrhizal trait data (fewer than 5% of all plant species; Brundrett 2009) we argue that strong generalizations about the predictability of plant mycorrhizal traits from plant taxonomy are unwarranted. In fact, rigid adherence to this assumption could lead to misdiagnosis when newly explored plant species diverge from expectations or a new type of mycorrhizal association is discovered for a plant species. Therefore, care needs to be taken in making predictions based on plant's taxonomic placement, particularly for AM and NM types or FM status, which are less phylogenetically conserved than ECM, ERM and ORM types (Maherali *et al.*, 2016; Bueno *et al.*, 2019). The amount of putative misclassification errors seems directly related to the level of taxonomy extrapolated, being higher within plant families than within genera (Bueno *et al.*, 2019). Still, in contexts where the available evidence is absent for a high number of species, plant phylogenetic relationships, which are not yet fully resolved (APG, 2016), could be used as a reasonable starting point for formulating hypotheses regarding missing plant mycorrhizal traits, but those hypotheses should be tested and not be assumed to be correct in advance. We advocate for a more flexible view of mycorrhizal symbioses, where plant and fungal partners, as well as our knowledge about them, are constantly evolving (Selosse *et al.*, 2018). Overall, more empirical research, observing and analyzing mycorrhizal fungal colonization of plant species in natural systems is needed to overcome limitations, improve our extrapolations and hypotheses, and ultimately build our knowledge of mycorrhiza on more solid ground.

### **Point 3. Constructing a 'standard reference'**

We agree with Brundrett and Tedersoo that detailed comparison with a critical review of knowledge accumulated in the field is necessary to contextualize new findings. However, there are unstated assumptions in the 'standard reference' proposed by Brundrett and Tedersoo that should be addressed in order for the field to advance. First, the binary evaluation ('correct' or 'incorrect') of earlier studies disregards differences in the conceptual frameworks or definitions used by those studies. We are not suggesting that existing databases are error free, but genuine errors in plant mycorrhizal traits need to be distinguished from variation that is generated by using alternate, but still valid definitions of the mycorrhizal symbiosis (see point 1). Given differences in the definitions used by researchers to assign mycorrhizal traits, opinions about the conclusions of prior studies without appropriate analytical and/or empirical support should not be treated as evidence that prior conclusions were incorrect (Table 4, Brundrett & Tedersoo 2019). In light of this, we do not think that researchers should be dissuaded from using curated databases, nor inherently disregard the output of analyses stemming from them without first examining the assumptions, objectives and definitions used by the authors. Second, it should be noted that the 'standard reference' cited in Brundrett and Tedersoo's viewpoint did not appear to be presented with detailed references or supporting information that could justify decisions about each family assignment to a mycorrhizal type or status (See point 4).

#### **Point 4. Towards a transparent database of mycorrhizal traits and future research**

Any methodological approach is expected to be independently reproducible. Reproducibility is enabled by a clear methodological description that includes the criteria, background information and evidence used to justify conceptual or analytical decisions ('Towards transparency', 2014; Powers & Hampton, 2018). This is crucial if methodologies are to be assessed, discussed and improved in light of both conceptual developments and the availability of new information. To achieve this, we suggest that the research community strives to build a standard reference that represents a review of empirical evidence for all examined species, providing references and evidence to support mycorrhizal trait assignment (by morphological and ideally by functional features), and highlighting known uncertainties and knowledge gaps for which more empirical research is needed.



We hope that the discussion prompted by Brundrett and Tedersoo's viewpoint draws attention to a lack of consensus about core concepts in mycorrhizal ecology and provides incentive for researchers to openly discuss the various challenges, and to agree on core concepts. Moving forward, we advocate that researchers are transparent about the different conceptual frameworks used to make plant mycorrhizal trait assignments in their studies, and that readers consider such frameworks when drawing inferences from those studies. To spur expansion of the empirical knowledge base, we advocate the development of more inclusive definitions of mycorrhizal symbiosis and suggest that researchers strive to reach consensus on suitable definitions that allow us to capture features of the symbiosis that we are just beginning to discover. In this respect, meetings and forums such as ICOM (International Conference on Mycorrhiza) are ideal. A concerted effort to account for this complexity in detailed data collation will pave the way for future multidisciplinary work and enable new discoveries, such as unearthing possibly novel roles of the symbiotic partners. This approach could encompass experimental and observational studies at molecular to global scales, and could lead us towards a more comprehensive understanding of the functional roles of mycorrhizal symbioses in ecosystems.

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### **Author contributions**

CGB and MM wrote the first draft, and all authors significantly contributed to revisions.

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